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PRESERVED LIVER FUNCTION AND LEUKOCYTE RESPONSE IN SUPERLETHAL --ETC(U)
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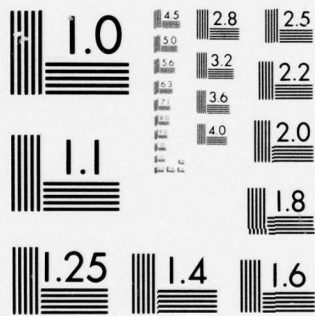
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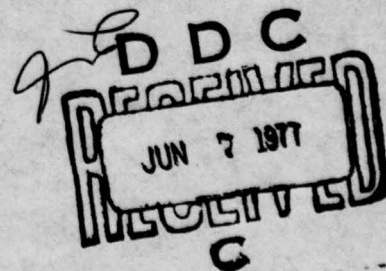
PRESERVED LIVER FUNCTION AND LEUKOCYTE RESPONSE
IN SUPERLETHAL ENDOTOXIC SHOCK

L. T. Archer, G. L. White, J. J. Coalson,
B. K. Beller, O. Elmore, and L. B. Hinshaw

Prepared for Publication
in
American Journal of Physiology

University of Oklahoma Health Sciences Center
Departments of Physiology & Biophysics, Surgery and Pathology
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⁶ PRESERVED LIVER FUNCTION AND LEUKOCYTE RESPONSE
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ABSTRACT

Recent studies reveal that endotoxin-pretreated awake dogs become markedly leukocytotic and survive superlethal endotoxin challenge without hypoglycemia. ^{sought} The purpose of this study was to determine if leukocytosis protects the liver and alters survival in endotoxin shock. Studies were conducted on awake, healthy dogs with the endotoxin group (N=5) injected LD 100 i.v. with 1/1,000 LD100 E. coli endotoxin on days 1 and 2, LD100 on day 3 and 2 x LD100 on day 4. The control group (N=6) received equal volumes of saline on days 1, 2 and 3, but on day 4 received 2 x LD100 endotoxin. All six saline-pretreated dogs died within 7 hr following superlethal endotoxin challenge. Since in parallel studies all endotoxin-pretreated dogs (n=11) lived 30 days, each animal in the experimental group was sacrificed at the time of its paired saline control's death for a comparison of liver pathology. Endotoxin experimental animals exhibited a marked leukocytosis (39,000/mm³) (p<0.001) on day 4 compared with saline-pretreated controls. At the time of death, liver enzymes arginase and SGPT were elevated (p<0.02) in the saline controls compared with endotoxin-pretreated dogs. Histological findings for saline-pretreated animals ranged from marked to massive hepatocellular necrosis, while endotoxin-pretreated dogs' liver pathology consisted of mild central lobular congestion and necrosis. Results suggest that leukocytosis protects liver function and enhanced survival in endotoxin shock.

INDEX TERMS: canine, endotoxin shock, liver function and leukocytes

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Alterations in carbohydrate metabolism have been recognized to play a pivotal role in host defense against shock; therefore, the hepatosplanchnic region may become the focal point determining circulatory and caloric homeostasis in shock. Recent studies have documented pathophysiologic manifestations in canine endotoxin shock or live E. coli septicemia including hypoglycemia, systemic hypotension, hepatosplanchnic dysfunction and death (3,18, 21,22). The progressively developing hypoglycemia has been proposed to result mainly from depressed liver and kidney function, particularly gluconeogenesis (2,11,14,18), although accelerated glucose uptake of blood has been implicated (2,22).

White blood cell (WBC) phagocytotic activity has been suggested as the primary factor responsible for the accelerated glucose uptake of blood following in vitro incubation of endotoxin and live E. coli organisms (22). Endotoxin is known to be efficiently phagocytized by polymorphonuclear leukocytes (6,7), and an associated increase in glucose utilization by neutrophils has been reported to occur (26,27). A recent report has shown circulating neutrophils to be of major importance in the clearance of bacterial organisms (25).

Recent results from this laboratory have shown that dogs become markedly leukocytotic when administered sublethal intravenous injections of endotoxin and subsequently survive a 2 x LD₁₀₀ endotoxin, in contrast to control animals challenged with the identical superlethal dose (23,29). It was hypothesized that effective phagocytosis in the buffy coat could spare the liver and reticuloendothelial system the stress of endotoxin detoxification and prevent depression of gluconeogenic function (23,29). Blood glucose concentrations were found to decrease during early leukopenia but returned to normal in the "protected" endotoxin-pretreated group, suggesting an association of leukocytosis with preserved liver function (23,29).

The purpose of the present study was to explore a possible protective role of the leukocytes against the lethal effects of shock and the altered gluconeogenic capacity of the liver.

METHODS

Eleven awake mongrel adult dogs of random sex, free of disease, were used in the present study. All animals were treated for intestinal parasites and dogs with heartworm microfilaria were eliminated. Animals were allowed a stabilization period of 3-6 weeks and only those with initial white blood cell (WBC) counts between 7,000 and 20,000/mm³ and hematocrits exceeding 37% were utilized. Experiments were designed to follow alterations in peripheral white blood cell (WBC) counts, rectal temperatures (T_R), serum insulin, blood glucose, arginase and serum glutamic pyruvic transaminase (SGPT) during administration of sublethal and superlethal doses of E. coli endotoxin (Difco, Detroit) without the influence of anesthetics. Other parameters monitored were concentrations of potassium, sodium, serum glutamic oxalacetic transaminase (SGOT), lactate dehydrogenase (LDH) and fractionated-lactate dehydrogenase (F-LDH), using a Technicon SMA 12/60 and 6/60 system "Chem 18" (Technicon Instruments Corporation, Terrytown, New York).

Dogs were divided into saline control and endotoxin experimental groups. The endotoxin group received sublethal doses of endotoxin of 0.003 mg/kg body weight on days 1 and 2 (i.e., 1/1,000 LD₁₀₀), 3 mg/kg on day 3 (i.e., LD₁₀₀) and a challenge dose of endotoxin of 6 mg/kg on day 4 (i.e., 2 x LD₁₀₀). The control group received equal volumes of saline on days 1, 2 and 3, but on day 4 received a 6 mg/kg challenge dose of endotoxin (i.e., 2 x LD₁₀₀). The LD₁₀₀ of E. coli endotoxin (3 mg/kg) had been previously established in a group of approximately 25 dogs. White blood cell counts, rectal temperatures

(T_R), insulin, glucose, arginase, SGPT, and Chem 18-surveyed agents were measured initially (i.e., before endotoxin or saline injection) during each of 4 days of observation. WBC, T_R and glucose values were measured at one and 6 hours post-injection on days 1, 2 and 3, while Chem 18 values were taken at one and 6 hours on day 1 but only at 6 hours on day 3. Recent studies from this laboratory documented that all dogs given sublethal injections of endotoxin survive a superlethal endotoxin shock (N=11), while 100% die when "pretreated" with saline and then challenged with the same superlethal dose of endotoxin (N=11) (23,29). Therefore on day 4 terminal blood samples were collected in both groups at the time of each saline-pretreated animal's death, the endotoxin-pretreated dog was sacrificed, post-mortem examinations were performed and tissues were evaluated by light and electron microscopy.

The WBC counts were measured with an automatic particle counter (Coulter ZF; Hialeah, Florida). Blood glucose concentrations were determined using a Beckman glucose analyzer (Beckman Instruments; Fullerton, Calif.) with an accuracy of ± 3 mg%, and rectal temperatures were obtained using a Tele-Thermometer probe (Yellow Springs Instruments; Yellow Springs, Ohio). Serum insulin values were determined by the Phadebas insulin test (Pharmacia; Uppsala, Sweden) as previously reported (2,3). Arginase and SGPT values were determined using Chem assay kits (Pitman Moore, Inc; Washington Crossing, N.J.). Blood samples were obtained by venipunctures of the jugular vein, then placed in vacutainers containing ethylenediamine-tetraacetic acid (EDTA; Beckton-Dickinson) or clot tube vacutainers. The injection of saline or endotoxin was by the intravenous route utilizing the cephalic vein. Results were analyzed using the t test for paired or unpaired data.

RESULTS

Table 1 presents WBC ($/\text{mm}^3$) and blood glucose (mg/100 ml) data obtained from animals receiving single sublethal injections of endotoxin on days 1, 2 and 3, and superlethal administrations of E. coli endotoxin on day 4. Daily values were obtained prior to injections of endotoxin in the experimental and control groups and are seen to reflect the effects of previous injections on days 2, 3 and 4. Significant leukocytosis ($p < 0.02$) is observed in the experimental group on days 2, 3 and 4, with an initial value of $38,700/\text{mm}^3$ at zero time on day 4, which is significantly elevated ($p < 0.001$) when compared with the control group. Following superlethal endotoxin challenge on day 4, mean glucose concentrations rose higher in the saline-pretreated group, although differences were not statistically significant due to variations between animals.

Results from Table 2 demonstrate significant increases in rectal temperatures ($^{\circ}\text{C}$) in dogs administered sublethal to lethal injections of endotoxin. Elevations in temperature were observed between one and 6 hours after sublethal injections of endotoxin on days 1-3 ($p < 0.05$) in contrast to the control animals in which rises were not seen. Superlethal challenge of endotoxin on day 4 elicited significant increases in temperature ($p < 0.02$) in both experimental and control groups.

Alterations in serum levels of arginase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase in response to sublethal and superlethal injections of endotoxin are presented in Table 3. Arginase levels are similar in both the endotoxin experimentals and saline controls for days 1-3, while on the fourth day after superlethal endotoxin challenge concentrations of the enzyme in the control dogs are significantly higher ($p < 0.02$) than in the endotoxin-pretreated group at both one and 6 hours post-endotoxin. On day 3 and at the day 4 initial sampling, concentrations of SGOT and SGPT were increased ($p < 0.025$) in the endotoxin-pretreated animals, but by 6 hours after

superlethal endotoxin challenge both SGOT and SGPT were markedly higher ($p < 0.005$) in the saline-pretreated control group.

Morphologic changes of the liver observed with light microscopy in 5 of 6 animals in the saline-pretreated group ranged from marked to massive central lobular necrosis throughout two-thirds or more of the individual lobules with central lobular congestion in the same 5 dogs. The 6 endotoxin-pretreated dogs exhibited minimal to mild central lobular congestion while two animals showed early minor central lobular necrosis. Liver sections from both groups contained minimal to mild numbers of polymorphonuclear leukocytes within the sinusoids.

Table 4

Table 4 illustrates changes in serum concentrations of LDH and F-LDH in response to endotoxin. On day 3, LDH was significantly increased ($p < 0.01$) in the control animals as compared with the endotoxin-pretreated group. Mean LDH concentration increased in the control group at the initial sampling on day 4 as compared with day 1, but this rise was not significant. On day 4, LDH and F-LDH values rose rapidly in the controls in response to superlethal endotoxin injection ($p < 0.01$) and notably exceeded the increases in the endotoxin-pretreated group at one and 6 hours after endotoxin ($p < 0.001$).

Table 5

Fluctuations in serum insulin and potassium concentrations in response to sublethal and superlethal injections of endotoxin are arrayed in Table 5. On day 4 at one hour after 2 x LD₁₀₀ endotoxin injection, serum insulin had decreased significantly ($p < 0.05$) in the endotoxin-pretreated group but had risen sharply ($p < 0.02$) in the control animals. The 254 μ U/ml concentration observed in the control group is markedly above ($p < 0.01$) the 10 μ U/ml value seen in the experimental animals. Mean insulin values remained at the high level in the control group although variations in individual animals rendered this increase statistically insignificant. By day 4, control potassium concentrations were lower in experimental animals ($p < 0.01$) than the initial

day 1 control values. In the control animals, potassium values increased from 4.5 to 8.7 mEq/L ($p < 0.01$) by 6 hours in response to superlethal endotoxin challenge, while the experimental group showed minimal changes. v

DISCUSSION

Results from the present experiments reveal a rapid leukopenia ($p < 0.05$) followed by a leukocytosis in response to sublethal and superlethal injections of endotoxin. In recent studies (23,29), all animals receiving daily sublethal injections of endotoxin survived superlethal challenges of endotoxin and were permanent survivors. Recent data (23,29), as well as results from the present study, show that animals not receiving sublethal endotoxin injections are very susceptible to the adverse effects of endotoxin and die in an average time of 6 hours. Normoglycemia was observed in the "protected" animals in contrast to hypoglycemia seen in the saline-pretreated control dogs at 5 1/2 hours post-endotoxin (29). The sharp contrast in blood glucose concentration and 100% survival versus 100% mortality observed between the two groups led us to speculate that the circulating neutrophil was playing a liver-sparing role. In order to test the hypothesis that the circulating leukocyte is the host's first line of defense and that the leukocytosis seen in endotoxin-pretreated dogs may protect the liver in its ability to perform gluconeogenesis, a battery of liver-specific enzymes as well as light microscopy of liver sections were studied.

The increased numbers of neutrophils seen in the present study may have effected an efficient and rapid clearance of endotoxin since Cline and co-workers (6) and Cohn et al. (7) have documented that neutrophils phagocytize endotoxin. Increased phagocytosis could account for the significant difference in the specific liver enzyme values observed between endotoxin- and saline-pretreated animals in response to 2 x LD₁₀₀ endotoxin injection. Six

hours after superlethal endotoxin challenge, SGPT values were 265 Units in the saline-pretreated animals compared with 100 Units ($p < 0.001$) in the endotoxin pre-injected dogs. Cornelius et al. (8) documented that elevated serum concentrations of glutamic pyruvic transaminase (GPT) are a sensitive specific test for liver necrosis since GPT is found in high concentrations in canine hepatic parenchymal cells. Arginase is found in significant concentrations within the liver of the dog (9), and therefore a marked elevation ($p < 0.05$) in plasma arginase activity from a mean of 9 at control to 296, as seen in response to $2 \times LD_{100}$ endotoxin in the saline controls, indicates a necrotic process within the liver. The "unprotected" saline group in the present study sustained more severe liver damage than the endotoxin-protected dogs in response to superlethal endotoxin challenge, as indicated by elevated serum arginase and GPT values as well as morphologic examination of liver tissues. These results are supportive of the hypothesis that the initial increased numbers of white cells ($38,700/\text{mm}^3$) through phagocytosis may be the most effective defense against the hepatic dysfunction observed in shock.

In the present study marked cellular damage was sustained by the saline-pretreated animals in response to superlethal endotoxin compared with endotoxin-pretreated dogs, since SGOT, LDH and F-LDH concentrations are significantly elevated at both one and 6 hours post-endotoxin ($p < 0.01$). Elevations in SGOT, LDH and F-LDH have been documented in the cases of diseases of the heart, liver and even skeletal muscle (4,30). An additive factor conceivably contributing to lethality may be the significant increases ($p < 0.01$) in serum potassium seen in the saline-pretreated dogs in response to $2 \times LD_{100}$ endotoxin compared with the endotoxin-pretreated animals. These elevations further support the present enzyme data suggesting notable tissue damage and reflecting a generalized loss of intracellular potassium.

Although other factors may have performed key functions in the survivability to endotoxin in the present study, the primary purpose of the present

study was to assay the role of leukocytes in the host-defense mechanism. It has been suggested that the host's primary defense mechanism against microbial infections depends upon phagocytosis and intracellular killing by polymorphonuclear leukocytes (25). The present study suggests the protective influences of leukocytosis as stimulated by sublethal injections of endotoxin. Leukocytosis associated with endotoxin has been reported to occur via entry of new leukocytes from the bone marrow into the circulation (19). Hollingsworth and Beeson (24) have shown that if rabbits are transfused with healthy leukocytes, injections of E. coli are cleared normally despite the fact that the transferred leukocytes have disappeared from the peripheral blood.

Since survivability has been associated with leukocytosis (23,29), there are possible therapeutic implications regarding the role of the white blood cell in endotoxin shock. Recent reports have described beneficial effects of transfused white blood cells as a treatment for septicemia in neutropenic patients (17) and dogs (12,13). Graw et al. (17) reported a significant increase in survival rate in patients with gram-negative sepsis when transfused with granulocytes.

In the present study rectal temperatures markedly increased ($p < 0.02$) in both saline- and endotoxin-pretreated dogs following superlethal endotoxin challenge although there were no significant differences in febrile response between the groups. Studies in rabbits administered endotoxin have shown that neutrophils release a pyrogen producing a febrile response (16). Other reports have produced evidence that endotoxin elicits fever by a direct action on the brain (15). Rabbits rendered leukopenic with nitrogen mustard and then given endotoxin exhibited a rapidly developing fever (16). Apparently the febrile response as observed in the present study played no role in determining lethality since the "protected" dogs spiked fevers equal to the "non-protected" animals; in fact, the febrile response may perform a beneficial

role in enhancing metabolic activities of the white cells (23) in the leukocytotic animals or improving hepatic blood flow on the basis of decreased blood viscosity, as well as through the release of a pyrogen derived from the leukocyte (16) or endotoxin itself (15).

Buchanan and Filkins (5) have documented in rats that any condition associated with elevated insulin secretion increases lethality in endotoxin shock, whereas lethality is reduced in situations of depressed insulin secretion. In the present study a marked hyperinsulinemia ($p < 0.02$) was observed in the saline-pretreated control dogs after superlethal endotoxin challenge, and all animals died. Hiebert et al. (20) compared insulin secretory rates (ISR) in dogs and monkeys subjected to hemorrhagic shock and found a six-fold increase in shocked dogs after glucose loading with no ISR response in monkeys. Glucose disappearance rates during intravenous glucose tolerance testing in the hypotensive dogs and monkeys were not different, suggesting that the alterations in glucose metabolism during shock in the dog might be mediated principally through peripheral factors of "insulin resistance", whereas the monkey was influenced more by altered insulin secretion (20). Insulin values of 250 $\mu\text{U/ml}$ in response to blood glucose concentrations of 140 mg% in the present study are excessive when compared with results from a study of burn patients in which Allison and co-authors (1) reported higher than normal insulin responses of approximately 250 $\mu\text{U/ml}$ to a glucose load of 450 mg%, suggesting an insulin resistance. A hyperglycemia of 140 mg% with an excessive insulin release was observed in the present study one hour post-endotoxin, but blood glucose remained at 142 mg% after 6 hours, further suggesting the presence of insulin resistance.

Normally, the early hyperglycemia of endotoxic, septic and hemorrhagic shock (3,5,10,14,20,21) has been attributed to sympathetically-stimulated glycogenolysis and alpha-adrenergic suppression of beta cell insulin release

from the pancreas (10). Data from the present study in endotoxic shock suggest that the dog escapes catecholamine-induced insulin suppression in accordance with data reported on the canine species in hemorrhagic shock (20).

Results from the present study show that the dog may be protected against the lethal effects of endotoxin shock ($2 \times LD_{100}$) by prior treatment with sublethal intravenous injections of endotoxin; data suggest that the febrile response is not a factor determining lethality and that the neutrophil may be the host's first natural line of defense against infection (28); and therefore leukocytosis plays a significant role in protecting the liver against damage, enhancing survival in endotoxin shock.

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TABLE 1. Effect of endotoxin on leukocyte and blood glucose concentrations in dogs

	Time (days)*				Time (hours)†	
	Day 1	Day 2	Day 3	Day 4	+1	+6
WBC (/mm ³)						
C‡	13,500	12,300	14,800	15,200	6,700	19,300
E‡	12,700	26,300	27,500	38,700	7,800	29,100
p§		.005	.001	.001		.05
Blood glucose (mg/100 ml)						
C	91	104	90	96	140	142
E	93	93	87	91	107	84
P						

Total of 11 dogs; 5 pretreated with endotoxin and 6 with saline. Experimental animals received sublethal doses of E. coli endotoxin; 1/1,000 LD₁₀₀ on days 1 and 2, LD₁₀₀ on day 3, and 2 x LD₁₀₀ (6 mg/kg) on day 4. The control group received volumes of saline equal to endotoxin solution on days 1, 2 and 3, and 2 x LD₁₀₀ on day 4.

*Initial measurements in each group on four consecutive days. Value for day 4 serves as the control measurement for the time designations of hours.

†Hours post-injection of 2 x LD₁₀₀ E. coli endotoxin. ‡ E = mean of

experimental group; C = mean of control group. § p = unpaired comparison between control and experimental groups.

TABLE 2. Effect of endotoxin on rectal temperature in dogs

Day	Group	Time (hours)*			
		0	+1	+3	+6
1	C†	38.3	38.4	38.3	38.3
	E†	38.1	38.7	39.5	38.8
	p [‡]			.001	.05
2	C	38.1	38.2	38.2	38.2
	E	38.2	39.0	39.5	38.6
	p		.001	.001	
3	C	38.1	38.2	38.1	38.1
	E	38.4	39.2	39.7	39.9
	p		.001	.001	.001
4	C	38.2	39.8	40.2	39.9
	E	38.8	40.3	40.0	39.7
	p	.05			

TR = °C. Total of 11 dogs; 5 pretreated with endotoxin and 6 with saline. Experimental animals received sublethal doses of *E. coli* endotoxin; 1/1,000 LD₁₀₀ on days 1 and 2, LD₁₀₀ on day 3, and 2 x LD₁₀₀ (6 mg/kg) on day 4. The control group received volumes of saline equal to endotoxin solution on days 1, 2 and 3, and 2 x LD₁₀₀ on day 4.

*Hours post-injection;
control group receiving saline only on days 1, 2 and 3.

†E = mean of experimental group; C = mean of control group.

‡p = unpaired comparison between control and experimental groups.

TABLE 3. Effect of endotoxin on arginase, serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in dogs

	Time (days)*				Time (hours)†	
	Day 1	Day 2	Day 3	Day 4	+1	+6
Arginase (IU/L)						
C‡	4	16	16	9	76	296
E‡	5	24	23	14	23	22
p§					.005	.02
SGOT (mU/ml)						
C	30	23	26	23	412	1,296
E	33	28	22	62	79	107
P				.01	.01	.005
SGPT (IU/L)						
C	51	49	41	38	208	264
E	72	71	73	151	140	101
P			.025	.01		.001

See legend to Table 1.

TABLE 4. Effect of endotoxin on lactate dehydrogenase (LDH) and fractionated-lactate dehydrogenase (F-LDH) in dogs

	Time (days)*				Time (hours)†	
	Day 1	Day 2	Day 3	Day 4	+1	+6
LDH (mU/ml)						
C $\frac{1}{2}$	103	151	204	211	1,251	3,259
E $\frac{1}{2}$	79	85	99	190	125	256
p§			.01		.001	.001
F-LDH (mU/ml)						
C	24	22	26	21	452	1,300
E	22	23	26	100	51	106
p				.05	.01	.01

See legend to Table 1.

TABLE 5. Effect of endotoxin on serum insulin and potassium concentrations in dogs

	Time (days)*				Time (hours)†	
	Day 1	Day 2	Day 3	Day 4	+1	+6
Insulin (μ U/ml)						
C $\frac{1}{2}$	13	18	32	20	254	278
E $\frac{1}{2}$	17	19	15	24	10	20
p $\frac{5}{8}$.01	
Potassium (mEq/L)						
C	4.5	4.5	4.6	4.5	3.2	8.7
E	4.6	4.4	4.4	4.1	4.0	4.6
P				.025	.02	.01

See legend to Table 1.

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13. ABSTRACT Recent studies reveal that endotoxin-pretreated awake dogs become markedly leukocytotic and survive superlethal endotoxin challenge without hypoglycemia. The purpose of this study was to determine if leukocytosis protects the liver and alters survival in endotoxin shock. Studies were conducted on awake, healthy dogs with the endotoxin group (N=5) injected i.v. with 1/1,000 LD ₁₀₀ <u>E. coli</u> endotoxin on days 1 and 2, LD ₁₀₀ on day 3 and 2 x LD ₁₀₀ on day 4. The control group (N=6) received equal volumes of saline on days 1, 2 and 3, but on day 4 received 2 x LD ₁₀₀ endotoxin. All saline-pretreated dogs died within 7 hr following superlethal endotoxin challenge. Since in parallel studies all endotoxin-pretreated dogs (N=11) lived 30 days, each animal in the experimental group was sacrificed at the time of its paired saline control's death for a comparison of liver pathology. Endotoxin experimental animals exhibited a marked leukocytosis (39,000/mm ³) (p<0.001) on day 4 compared with saline-pretreated controls. At the time of death liver enzymes arginase and SGPT were elevated (p<0.02) in the saline controls compared with endotoxin-pretreated dogs. Histological findings for saline-pretreated animals ranged from marked to massive hepatocellular necrosis, while endotoxin-pretreated dogs' liver pathology consisted of mild central lobular congestion and necrosis. Results suggest that leukocytosis protects liver function and enhances survival in endotoxin shock.			

